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Biomarkers of DNA damage in COPD patients undergoing pulmonary rehabilitation: Integrating clinical parameters with genomic profiling

Patrizia Russo[a,1], Palma Lamonaca[a,1], Mirta Milic[b], Emilio Rojas[c], Giulia Prinzi[a], Vittorio Cardaci[d], Laura Vitiello[e], Stefania Proietti[f], Alessia Santoro[a], Carlo Tomino[f], Massimo Fini[f], Stefano Bonassi[a,g,*]

[a] *Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Pisana, Roma, Italy*
[b] *Institute for Medical Research and Occupational Health, Zagreb, Croatia*
[c] *Departamento de Medicina Genòmica y Toxicologìa Ambiental, Instituto de Investigaciones Biomédicas, Universidad Nacional Autònoma de México, Ciudad Universitaria, Mexico*
[d] *Unit of Pulmonary Rehabilitation, IRCCS San Raffaele Pisana, Rome, Italy*
[e] *Unit of Flow Cytometry IRCCS San Raffaele Pisana, Rome, Italy*
[f] *Scientific Direction, IRCCS San Raffaele Pisana, Rome, Italy*
[g] *Department of Human Sciences and Quality of Life Promotion, San Raffaele University, Rome, Italy*

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease characterized by severe respiratory symptoms. COPD shows several hallmarks of aging, and an increased oxidative stress, which is responsible for different clinical and molecular COPD features, including an increased frequency of DNA damage. The current pharmacological treatment options for COPD are mostly symptomatic, and generally do not influence disease progression and survival. In this framework, pulmonary rehabilitation is the most effective therapeutic strategy to improve physical performance, reducing hospital readmissions and mortality. Response to rehabilitation may greatly differ among patients calling for a personalized treatment. In this paper we will investigate in a group of COPD patients those variables that may predict the response to a program of pulmonary rehabilitation, integrating clinical parameters with cellular and molecular measurements, offering the potential for more effective and individualized treatment options. A group of 89 consecutive COPD patients admitted to a 3-weeks Pulmonary Rehabilitation (PR) program were evaluated for clinical and biological parameters at baseline and after completion of PR. DNA fragmentation in cryopreserved lymphocytes was compared by visual scoring and using the Comet Assay IV analysis system. The comparison of DNA damage before and after PR showed a highly significant increase from 19.6 ± 7.3 at admission to 21.8 ± 7.2 after three weeks of treatment, with a significant increase of 2.46 points ($p < 0.001$). Higher levels of DNA damage were observed in the group of non-responders and in those patients receiving oxygen therapy. The overall variation of %TI during treatment significantly correlated with the level of pCO_2 at admission and negatively with the level of IL-6 at admission. Measuring the frequency of DNA damage in COPD patients undergoing pulmonary rehabilitation may provide a meaningful biological marker of response and should be considered as additional diagnostic and prognostic criterion for personalized rehabilitation programs.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a global preventable, but progressive lung disease characterized by severe respiratory symptoms, including dyspnea, breathlessness, cough, sputum production, and recurrent infections. Activities of daily living, especially walking, may be compromised in these patients for these patients, and they often experience (re)hospitalization [1,2]. COPD is characterized by large heterogeneity both in disease presentation and in grades of impairment with serious extra-pulmonary components (comorbidities) with considerable variability between individuals [1]. Patients experience acute exacerbations (AECOPD) frequently, a

* Corresponding author at: Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Pisana, Via di Val Cannuta, 247, 00166 Rome, Italy.
E-mail address: stefano.bonassi@sanraffaele.it (S. Bonassi).
[1] These authors contributed equally to the work.

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condition which contributes to high rates of admission to emergency departments with high costs for national health systems [2]. These latter patients are often old, affected by several comorbidities, and are burdened by a high prevalence of adverse outcomes [3].

COPD is one of the leading causes of morbidity and mortality worldwide [4]; in Italy affects 3.5 million people, accounting for 55% of deaths/year among respiratory diseases (3rd cause of death). The incidence, in the general population, is still increasing and with an ageing population, this number is expected to raise more [5]. The onset of the disease is complex; multiple causes beyond smoking contribute to the development of COPD such as environmental exposure, age-related degenerative changes, and genetic factors [6–9]. Thus, in elderly people COPD is the result of different gene-gene (G x G) and gene-environment (G x E) continuous interactions that happen in the life course of a single person [6]. COPD occurring at an earlier age than expected may result from the interaction of inherited factors and environmental exposure [10]. Genetic variants (SNPs) are associated with COPD susceptibility [11], severity (p53, MDM2) [12], airway hyperactivity, FEV [13], and therapeutic response (FKBP5) [9]. COPD shows several hallmarks of aging [6,14], and an increased oxidative stress, which is responsible of different COPD features [14,15]. Smoking and environmental pollutants represent the main source of exogenous oxidants and reactive oxygen species (ROS) [16]. In turn ROS recruit inflammatory cells such as macrophages and neutrophils into the lung, which then produce endogenous ROS. Increased oxidative stress is related to a decrease expression of the nuclear factor-erythroid 2 related factor 2 (NRF2), NADPH oxidases (NOX), myeloperoxidase (MPO) and superoxide dismutase (SOD). ROS cause reversible and irreversible protein modifications and RNA, DNA, and mitochondrial DNA (mtDNA) damage [17].

In clinical studies, the frequency of DNA damage, measured in peripheral blood lymphocytes of COPD patients with the comet assay was higher when compared to control individuals [18]. However, DNA damage did not correlate with age, smoking habits, levels of airflow obstruction or COPD severity [18]. Similar results, showing a significant increase in DNA damage both in smoking COPD patients and in COPD patients previously exposed to biomass fuel were published by Ceylan and colleagues [19].

DNA damage is normally repaired by the efficient DNA repair machinery, however in COPD it has been hypothesized a failure in repairing double-stranded DNA breaks [20], since apurinic/apyrimidinic (AP) sites, indicating sites of base excision and repair, do not increase in COPD tissue. Unrepaired DNA damage may lead to genomic instability and cellular senescence [21]. The concentration of eosinophils, a possible exacerbation marker [22], is involved in the mechanisms of DNA repair in the lung [23]. Eosinophils are able to repair methyl methanesulfonate (MMS)-induced single-strand DNA breaks and mitoxantrone-induced double-strand DNA breaks, evaluated using alkaline and neutral single-cell gel electrophoresis (comet assay), respectively [23]. COPD is linked to increased mitochondrial ROS production, decreased intracellular antioxidants, and reduced numbers of mitochondria [24] The mitochondrial stress markers Parkin and PTEN-induced protein kinase-1 (PINK1) are increased in COPD patients [25].

COPD is generally associated with other non communicable diseases (NCDs), and several of them are characterized by features of accelerated aging [14]. Ischemic heart disease, type 2 diabetes, metabolic syndrome, and metabolic bone disease may share common molecular pathways with COPD, including an increased oxidative stress and the consequent DNA damage [26–29].

The current pharmacological treatment options for COPD are limited, mostly symptomatic, and generally do not influence disease progression and survival [30]. The scenario is more complicated by the common presence of comorbidity [31]. In this framework pulmonary rehabilitation is the most effective therapeutic strategy to improve shortness of breath, health status, and exercise tolerance, reducing in the end hospital readmissions and mortality [2]. However, response to rehabilitation may greatly differ among patients, even with similar clinical and demographic characteristics. In addition to traditional demographic, social, psychological, and clinical predictors, variability may be attributed to individual genetic and epigenetic variations that influence the response to treatment. The comprehensive understanding of the genomic profile is a critical step forward for the construction of a personalized therapeutic approach and will make the difference within the rehabilitative framework as a fundamental tool for the recovery of disability and the improvement of quality of life (QoL). The recent precision medicine approach, the so called ‘Rehabilomics’, represents a translational framework for programs of personalized rehabilitation, focused on linking personal biology to the bio-psychosocial constructs [32–36].

In this paper we will investigate in a group of COPD patients those variables that may predict the response to a program of pulmonary rehabilitation (PR), integrating clinical parameters with cellular and molecular measurements, offering the potential for more effective and individualized treatment options.

2. Materials and methods

2.1. Study design and participants

The study was submitted and approved by the ethics committee of the IRCCS San Raffaele Pisana (Prot. 15/2013), and all participants gave separate consent to participate to the study. This interventional non pharmacological study was conducted in 89 consecutive enrolled patients aged 70 years or older suffering from GOLD 3–4 COPD [2], and admitted to the Pulmonary Rehabilitation (PR) Unit of the IRCCS San Raffaele Pisana between January 2013 and December 2015 for a comprehensive 3 weeks PR program. All patients were part of a larger study on the application of Systems Medicine approaches in real life, and received an extensive multidisciplinary and multidimensional assessment [37,38].

All patients received daily inhalation treatment with corticosteroids (beclomethasone dipropionate [$C_{28}H_{37}ClO_7$] (0.4 mg/ml), in combination with bronchodilators, i.e., SABA (Short Acting Beta2 Agonists in our patients Salbutamol) and SAMA (Short-Acting Muscarinic Antagonist in our patients anti sub type M3:Ipratropium bromide) for 3 weeks. Exacerbations were treated with Prednisone [$C_{21}H_{26}O_5$] (5–25 mg) or betamethasone [$C_{22}H_{29}FO_5$] (1.5 mg) or with Methylprednisolone [$C_{22}H_{30}O_5$] (20 mg).

The following patient characteristics were measured at baseline and/or after completion of PR as previously described [38]: 1.Demographics; 2.Medical history and life-style; 3.Body Mass Index (BMI); 4.Health-related QoL evaluated using the St. George's Respiratory Questionnaire (SGRQ, scores range from 0 to 100). 5.QoL evaluated using the Activities of Daily Living (ADL, scores range from 0 to 6); the Instrumental Activities of Daily Living (IADL, scores range from 0 to 6); the 36-Item Short Form Health Survey General, and Mental Health (SF-36,scores range from 0 to 100). 6.Use of Long Term Oxygen Therapy (LTOT); 7. Strumental evaluation [spirometry, pulse oxymetry, blood pressure, electrocardiography (ECG), heart beats]; 8.Disease-specific respiratory status using the Medical Research Council (MRC) (scores range from 0 to 5), the Barthel and the Borg scales for assessing dyspnea (scores range from 0 to 10); and the Maugeri Foundation Respiratory Failure Questionnaire (MRF 26, scores range from 0 to 5). 9.Functional exercise capacity using the six Minutes Walking Test (6MWT) [39]; 10.Cognitive and psychological clinical alterations/disorders; 11.Hematology tests

Peripheral blood samples were collected and stored at -80 °C at hospital admission and after 3 weeks of PR.

2.2. Comet assay

Blood diluted 1:1 with PBS (Ca/Mg free) transferred in leucosep

tubes was centrifuged at 800 x g for 15′ at room temperature, without acceleration and deceleration. The lymphocyte ring was transferred to a falcon tube, washed with 10 ml of PBS Ca/Mg free) and centrifuged twice at 250 × *g* for 10 min at 4 °C. The pellet was resuspended in 1 ml of freeze-medium (Synth-a-freeze cryopreservation medium, Thermo Fisher Scientific), splitted into 2 vials, frozen at -80 °C, and then stored in liquid nitrogen.

Cryopreserved lymphocyte aliquots are thawed on ice, immediately diluted in 5 ml PBS and centrifuged for 5 min at 180 x g to remove the cryopreservation medium. The pellet was resuspended in PBS and cell suspension was processed for the alkaline comet assay.

Cells were suspended in 0.5% (w/v) low-melting point agarose and sandwiched between a lower layer of 0.5% (w/v) normal-melting point agarose and an upper layer of 0.5% (w/v) low-melting point agarose on microscope slides. The slides were then immerse in a lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, pH 10) containing 10% DMSO and 1% Triton X-100, overnight at 4 °C.

At lysis completion, the slides were place in a horizontal gel electrophoresis tank with fresh alkaline electrophoresis buffer (200 mM Na_2EDTA, 10 N NaOH pH = 13) and left in the solution for 10 min at 4 °C to allow the DNA to unwind and the alkali-labile sites to express. Electrophoresis was carried out at 4 °C for 10 min, 25 V (0.8 V/ cm) and 300 mA. After electrophoresis, the slides were neutralized for 5 min in Tris 0.4 M (pH 7.5), fixed for 5 min in absolute ethanol and air-dried at room temperature. Immediately before scoring, slides were stained with 20 μg/mL ethidium bromide. Individual cells were visualized at 40x magnification under an AXIO Imager-Z2 (Carl Zeiss) microscope with fluorescence attachments and the DNA damage was determined. To evaluate DNA migration, 100 cells were scored for each slides. The data are categorized into five categories according to the Olive tail moment score as described by Collins [40]. The total number of cells in each category were counted and multiplied by an assigned value 0–4 according to the damage class. The sum of all the categories was calculated, divided by the number of cell scored and considered as the damage index. The overall score was expected to vary between 0 and 4 arbitrary units.

Scoring and analysis of slides was also performed using the Comet Assay IV analysis system (Perceptive Instruments Ltd., UK), connected with an epifluorescence microscope (Olympus BX51, Tokyo, Japan). A total of 100 comets per treatment were scored on duplicate slides, at 200× magnification. DNA damage was estimated based on tail length and tail intensity (DNA% in the comet tail), measured by the Comet Assay IV software.

2.3. IL-6 quantification

The levels of IL-6 in the plasma samples were determined by Enzyme-Linked Immunosorbent Assay (ELISA) protocols according the manufacturer's recommendations using the ELISA Development Kit of Peprotech Inc, New Jersey, USA.

2.4. Statistical analysis

Descriptive analyses of epidemiological, clinical, and biological data were presented as mean ± standard deviation for continuous variables, and as proportion for categorical variables. The comparison of results at admission and after pulmonary rehabilitation was performed with the student's *t* test for paired data, after log transformation of original data. Multiple regression analysis was performed fitting log transformed values of tail intensity and adjusting for actual confounders, age and sex, and fixed effects (DNA damage at baseline). Potential confounders were selected from significant variables from univariate analyses. A statistically significant value was considered when p ≤ 0.05. All statistical analyses were performed using the statistical software SPSS IBM SPSS Statistics 25.0 [41] and STATA [42].

Table 1
Demographics, Life Style, and Clinical Features of COPD Patients (Patients n = 89).

Variables	
Gender	
Males	40 (44.9%)
Females	49 (55.1%)
Age *(years)*	72.42 ± 8.75
Education *(years)*	8.9 ± 3.88
Marital Status	
Single	4 (4.5%)
Married	49 (55.0%)
Divorced/widow	36 (40.5%)
Current Smokers	15 (18.8%)
Occupational Status	
Active	14 (17.7%)
Retired	57 (72.1%)
Housewife	8 (10.2%)
BMI	27.2 ± 7.6
Living alone	20 (22.5%)
Therapy with O_2	30 (33.7%)
MMSE	27.12 ± 2.82
MoCa	26.0 ± 3.58
SAS	28.88 ± 8.54
BDI-II	13.93 ± 8.17
SF-36 *General Health*	72.24 ± 12.28
SF-36 *Mental Health*	62.44 ± 9.99
CIRS-*comorbidity*	2.58 ± 1.43
CIRS-*severity*	1.60 ± 0.23

3. Results

A total of 89 elderly patients (mean age ± SD: 72.4 ± 8.8 years) with 3–4 GOLD stage COPD were recruited. The majority of patients were female (55.1%). Demographic characteristics, life style, lung functions and other clinical features at baseline are reported in Table 1. COPD patients are moderately overweight [43], do not report major cognitive impairment, have an impaired disease-specific health status, a reduced exercise tolerance, and 33.7% of them are under oxygen supplementation. Patients are moderately depressed and anxious. The severity of their health status at admission is well described by the performance in the 6MWT, with COPD patients walking only 94.0 ± 83.7 m. The wide variability of this estimate reflects the heterogeneity of the clinical response in this group.

At the end of the 3-weeks PR program all measures of respiratory outcome showed significant improvements (Table 2). Specifically, highly significant changes were observed for lung functions (FEV1, MRC, Borg), global functioning (6MWT, Barthel), laboratory testing (pH, pCO_2, SpO_2). Table 3 shows changes in hematological parameters after PR. The number of neutrophils decreases while the number of lymphocytes increases. The ratio of these two parameters (NRL) decreased significantly (p < 0.01) suggesting a reduction of

Table 2
Clinical respiratory parameters before and after pulmonary rehabilitation (Patients n = 89).

	Before PR *(x ± SD)*	After PR *(x ± SD)*	Δ%TI	p value
6MWD *(meters)*	94.04 ± 83.73	187.19 ± 133.81	93.15	< 0.001
FEV1*(%)*	52.57 ± 27.60	60.80 ± 29.72	8.24	0.012
MRC	4.0 ± 0.00	3.38 ± 0.55	−0.62	< 0.001
Borg	7.89 ± 0.92	5.20 ± 2.48	−2.68	< 0.001
SGRQ	48.42 ± 16.47	42.88 ± 14.86	−5.54	0.004
Barthel	69.74 ± 21.92	86.16 ± 16.31	16.42	< 0.001
MRF	73.01 ± 14.23	48.91 ± 17.92	−24.11	< 0.001
pH	7.42 ± 0.03	7.43 ± 0.03	0.01	0.017
pO_2*(mmHg)*	80.91 ± 20.03	79.13;10.90	−1.78	N.S.
pCO_2*(mmHg)*	44.87 ± 7.92	43.22 ± 8.37	−1.65	0.083
SpO_2*(mmHg)*	94.98 ± 2.60	95.89 ± 1.63	0.91	0.005

Table 3
Clinical pathology assessment of patients before and after rehabilitation (Patients n = 89).

	Before PR (*x ± SD*)	After PR (*x ± SD*)	Δ%TI	p value
CRP	0.88 ± 1.39	1.21 ± 1.74	0.33	N.S.
WBC*	10.28 ± 4.08	9.80 ± 3.20	−0.48	N.S.
Neutrophils	7.68 ± 3.67	6.73 ± 3.15	−0.95	0.023
Lymphocytes	1.72 ± 0.91	1.97 ± 0.94	0.25	0.011
Monocytes	0.73 ± 0.40	0.68 ± 0.30	−0.05	N.S.
Eosinophils	0.09 ± 0.12	0.12 ± 0.11	0.03	N.S.
Basophils	0.025 ± 0.02	0.03 ± 0.03	0.005	N.S.
IL-6	81.5 ± 124.4	110.8 ± 138.3	29.3	0.024

* 10^3/ml; CRP: C-reactive Protein; WBC: White Blood Cells.

inflammation, although the level of IL-6 increases after PR (from 81.5–110.8, $p < 0.05$).

DNA damage evaluated by comet assay, was analyzed using different endpoints, i.e., visual score, head length, tail length, head intensity, tail intensity, tail moment, and total intensity. All the endpoints were highly correlated and gave overlapping results (data not shown) as already suggested and demonstrated by other authors [40], and therefore we reported only data of tail intensity (%TI) value analysis.

To evaluate the effect of rehabilitation on DNA damage %TI values are expressed as the difference before and after treatment (Δ%TI). The comparison of %TI value before and after PR showed a highly significant increase from 19.6 ± 7.3 at admission to 21.8 ± 7.2 after three weeks of treatment. Changes in the level of DNA damage were compared after stratification for the two parameters that were expected to be the strongest predictors of DNA damage, i.e., the presence of treatment with oxygen therapy and the response to treatment. Results reported in Table 4 confirmed the role of these parameters in influencing DNA damage associated with the physical exercise although the interaction term did not reached statistical significance. The group of non-responders showed a 22.1% increase of %TI after PR as compared to baseline damage, which is much larger that the same effect observed in responders (8.9%), both difference reaching statistical significance. Similarly, subjects undergoing oxygen therapy showed a stronger effect of physical treatment on genome stability, showing a 14.7% increase of %TI after treatment ($p < 0.009$) *vs* 11.5% in subjects not receiving oxygen ($p < 0.002$). It should be noted that subjects treated with oxygen had a higher background level of DNA damage at baseline ($p < 0.05$ after PR), confirming as this parameter was firmly associated with the extent of DNA damage. A further subgroup analysis evaluating DNA damage in subject receiving oxygen therapy and not responding to the treatment showed the largest effect (from 19.4 to 24.6, that is a 26.9% increase with respect to baseline, $p < 0.05$). The overall variation of %TI during treatment significantly correlated with the level of pCO_2 at admission and negatively with the level of IL-6 at admission. On the other hand, this parameter did not correlate with respiratory outcomes, with gender, age class, smoking habit, BMI and years of education (data not shown). A multiple regression analysis was fitted to the pre-post PR difference of %TI, including age and sex, %TI at baseline as fixed effect, and testing clinical, epidemiologic, and laboratory parameters as confounders or effect modifiers. The final model did not show any variables significantly affecting the increase of DNA damage observed after treatment, although subjects undergoing oxygen therapy showed a borderline increase in the final model (β = 2.259, 95% CI -0.500:5.018; $p < 0.10$).

Table 4
Comparison of the level of DNA damage (%TI) before and after rehabilitation in selected subgroups (Patients n = 89).

	N.	%TI before PR (*x ± SD*)	%TI after PR (*x ± SD*)	ΔTI	p value
All patients	89	19.6 ± 7.3	21.82 ± 7.20	2.46	0.0001
Oxygen supplementation					
Yes	28	21.4 ± 8.6	24.5 ± 8.6	3.14	0.0086
No	56	18.4 ± 6.4	20.5 ± 6.0	2.11	0.0020
Response to PR					
Yes	58	20.00 ± 8.2	21.8 ± 6.8	1.78	0.0040
No	26	17.9 ± 4.5	21.9 ± 8.2	3.97	0.0014

4. Discussion

The results of the present study showed a remarkable improvement in most clinical and functional parameters as a consequence of pulmonary rehabilitation. Our results indicate also - in agreement with Maluf et al. [18] - an increased level of DNA damage in peripheral blood lymphocytes of patients with COPD after three weeks of treatment. The technique of the comet assay resulted extremely sensitive in recognizing the effect of physical rehabilitation on genome stability.

It has been reported that in healthy adult the occurrence of DNA single strand breaks (SSBs), is correlated with the aerobic capacity (evaluated after 6MWT) [44], while in a group of elderly participating to a 6 months program of physical training no increase of DNA SSBs was reported [45]. The influence of the exercise regimen used is extremely important in the induction and persistence of DNA damage, as suggested by different studies comparing untrained individuals with well-trained athletes (often with the comet assay) [46–49]. It has been reported that DNA SSBs and oxidative DNA damage (FPG-sensitive sites) decrease after 16 weeks of combined physical exercise training. The lack of significant changes in the DNA repair activity (OGG1 activity enzyme) of these subjects was possibly explained by an increase in antioxidant and metabolic efficiency [50]. It has been shown that in patients with COPD, there is a significant increase in ROS-induced DNA damage, evaluated by the comet assay, immediately after the maximal exercise level, not returning to baseline values after 4 h. In contrast after 8 weeks of rehabilitation there is no significant increase of ROS-induced DNA damage after the submaximal exercise test. The authors of this study attributed these results to an improved training status of the patients with COPD [51]. In our study patients received only 3 weeks of rehabilitation and it is possible that they do not reached yet an improved training status sufficient to counteract the ROS-induced DNA damage.

While we did not find any correlation between levels of airflow obstruction, COPD severity, and DNA damage, as reported also by Maluf et al [18], the increase of DNA damage observed during rehabilitation was significantly correlated with the level of pCO_2 at baseline. The level of pCO_2 at admission is associated with a moderate acidosis (> 40 mmHg = acidosis, according to reference no. [52]) that tends to normalize after rehabilitation. When the blood gas carbon dioxide level is over 45 mmHg, this condition is generally defined as hypercapnia [53]. Hypercapnia occurs as a consequence of poor alveolar ventilation and impairs alveolar fluid reabsorption (AFR) by promoting Na, K-ATPase endocytosis [54]. “In vitro” comet assay showed that CO_2 induces oxidative stress and cell DNA damage, leading to p53 up-regulation [55].

In our study multiple regression analysis showed that the variation of DNA damage during the rehabilitation is negatively correlated with the level of IL6 at baseline. It is known that there is a strong association between IL-6 and COPD affection status, airflow limitation, and emphysema progression [56,57] as well as with the severity of COPD [58]. IL-6, a 26 kDa 184 amino acid multifunctional glycoprotein, belong to a family of pro-inflammatory cytokines. IL-6 is produced by different stromal and immune cells, and is associated with pulmonary and extra-pulmonary inflammatory diseases (see reviews [57]. It has been reported that IL-6 inversely contributes to the changes in FEV1 (percentage predicted) [57]. Therefore, increased IL-6 levels are associated with mortality and also with reduced exercise tolerance at baseline and

after three years [59]. Indeed basal IL-6 is considered a marker of poor outcome [60]. In the general healthy population the plasma levels of IL-6 increase in response of a single session of physical exercise and these elevations seem to promote an anti-inflammatory environment [61–63], thus regular training decreases the basal plasma level of IL-6 [64–66]. Our results - in agreement with Dorneles et al [67] – showed an increased level of IL-6 after 3 week of pulmonary rehabilitation. Literature data show that more than 24 weeks of physical exercise, decreases dramatically the IL-6 level [68,69]. Our results showed also a reduction of the NRL after rehabilitation. The NLR is a potential marker of COPD progression and outcomes and is considered a valuable predictor of COPD exacerbations and mortality [70,71].

Actually, PR is believed as one of the most effective therapies for COPD and other respiratory disorders since its benefit is uncontestable resolving clinical problems caused by breathlessness and fatigue. Moreover, pulmonary rehabilitation helps patients to become more active in the management of their health problems [72]. PR is a multidimensional intervention including drug therapy (bronchodilators and corticosteroids), physical exercise therapy, disease education, changing behavior (i.e. smoking quitting, physical activity, diet) and psychological support [72–74]. Although the uncontestable benefits of PR, the patient response is different when a large sample of patients is examined [39] highlighting the need of a personalized approach.

The NIH's recent research plan on rehabilitation [75] states that part of its translational science goals will be to advance the understanding of precision medicine approaches relevant to rehabilitation medicine and, to characterize biomarkers associated with specific injuries, illnesses, or disorders that are prognostic or guide prescription of rehabilitation interventions. In addition, NIH 2017 rehabilitation guidelines emphasize the need to increase of the quality of evidence for rehabilitation interventions increasing focus on the design, dose, intensity, timing, and mechanisms.

Under this perspective we reported previously the use of micronuclei frequency evaluated in lymphocytes (as a marker of fixed or accumulated genetic damage) as a biomarker of frailty. Indeed there is a direct relationship between frailty in older adults and genome instability [76] as well as oxidative stress [77]. Frailty is a condition that involves increased risk of disability, mortality, morbidity, hospitalization, and institutionalization mortality in older adults [78]. The parallel approach on COPD using the extent of DNA damage as measured with the comet assay may successfully address NIH recommendations.

5. Conclusion

According to our results, measuring the frequency of DNA damage in COPD patients undergoing pulmonary rehabilitation may provide a meaningful biological marker of response and should be considered as additional diagnostic and prognostic criterion, to be considered for personalized rehabilitation programs. Furthermore, the well substantiated presence of DNA damage and genomic instability in the ageing process and in most non communicable diseases, calls for further investigation on this issue in a wider perspective.

Declarations of interest

None.

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